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Avian Influenza Virus Characteristics, Epidemiology, and Pathogenesis in Poultry in Egypt

Walaa A. Tourky [1], Mahmoud Ibrahim [2], Ahmed Said [2], Rania F. EL Naggar [1, 3], and Mohammed A. AboElkhair [1, *]

[1]Department of Virology, Faculty of Veterinary Medicine, University of Sadat City, Minoufiya, Egypt
[2]Department of birds and rabbit medicine, Faculty of veterinary medicine, University of Sadat city, Menufiya, Egypt
[3]Division of Biomedical and Life Sciences, Faculty of Health and Medicine, Lancaster University, Lancaster LA1 4YG, UK

Abstract: Since it was brought to Egypt in 2006, avian influenza virus has full-fledged to be one of the most prevalent and dangerous respiratory viruses. Over time, the avian influenza viruses have undergone significant evolution and grown increasingly due to antigenic drift and shift with difficulty to control. In recent years, Egypt's chicken industry has struggled to contain this disease due to maintenance of circulation of old avian influenza virus subtypes and development of new subtypes among Egyptian poultry flocks. Additionally, circulation of other respiratory viral pathogens, for example, infectious bronchitis virus & velogenic Newcastle disease virus strains have complicated process of control of AIV infection in Egypt. In the current review, we shed the light on the virus pathogenesis including effect of viral proteins and epidemiology with special focus on the significance of diverse infections between avian influenza virus along with additional respiratory pathogens, which generate more deadly symptoms and rapid bird deterioration.

***Keywords*:** Avian influenza virus; Evolution; Pathogenesis; Epidemiology; Mixed infection

*Correspondence: Mohammed A. AboElkhair
Department of Virology, Faculty of Veterinary Medicine, University of Sadat City, Minoufiya, Egypt
Email: mohamed.abouelkhair@vet.usc.edu.eg

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1.Introduction:

Avian influenza is a severe & deadly virus that has a significant threat to the global poultry business (Swayne et al., 2020; Salaheldin et al., 2022). The continuous genetic alteration of avian influenza viruses (AIV) hinders efforts to control their spread by generating new viral strains often (Dalby, 2016). One of the most significant turning moments in Egypt's history of avian influenza viruses occurred in 2006 when the 1st H5N1 strain landed there. While the H5N8 strain was first found in 2016, the H9N2 strain was first identified in 2011. The presence of these three strains in Egypt constitutes an ongoing danger to the poultry industry because they are among the most deadly and highly contagious respiratory illnesses, with high fatality rates (Aly et al., 2008; El-Zoghby et al., 2012; Selim et al., 2017; Yehia et al., 2018; Shehata et al., 2019). It is believed that avian influenza viruses originate naturally in waterfowl as well as migrating birds aid in the virus's international transmission among states (Kandeil et al., 2017). There has been a substantial increase in the mortality rates of birds with respiratory diseases. According to Roussan et al. (2008b) and Haghighat-Jahromi et al. (2008), this has been connected to a higher death rate brought on by other types of microorganisms present as mixed infections with avian influenza. The key subjects of this study are the molecular structure with special focus on viral proteins and their effects on virus pathogenesis, mixed infection with other microorganisms, virus evolution, and recent updated on the epidemiology of the virus.

2. AIV classification and nomenclature:

The pathogen responsible for avian influenza disease is influenza virus that is classified under Realm: Riboviria, Kingdom: Orthornavirae, Phylum: Negarnaviricota, Subphylum: Polyploviricotina Class: Insthoviricetes, Order: Articulavirales, Family: Orthomyxoviridae, Genus: Alphainfluenzavirus Species: Influenza A virus (ICTV, 2022).

Based on the proteins of M1 & NP, Influenza viruses are categorized into a total of 4 groups by the agar gel immune diffusion assay (AGID): influenza A, which includes all avian influenza viruses; influenza B, which mainly affects humans but has also impacts seals; influenza C, which affects swine in addition to humans; & finally, influenza D, which was recently found to be originating from cows (Maclachlan, 2016). A virus can be separated into several subtypes depending on the antigenicity of the influenza surface proteins hemagglutinin (HA) as well as neuraminidase (NA). There are eighteen HA antigens designated from H1 to H18, & eleven N antigens labelled from N1 to N11. With the exception of H17N10 in addition to H18N11 subtypes, which were discovered in bats, all subtypes have been identified in bird species (Tong et al., 2013).

Human influenza viruses with HA & neuraminidase subtypes are often limited to H1, H2, H3, N1, & N2. Conversely, viruses for influenza that propagate poultry have the capacity to contain almost all hemagglutinin and NA subtypes (Nicholson, 2003). Based on their phenotypic traits, subtypes H5 & H7 of the avian influenza are categorized as HPAI (highly pathogenic avian influenza), while subtypes H1–16 is classified as LPAI (low pathogenic AIV). This classification depends on in vivo testing, specifically the capacity to induce severe fatal sickness in chickens through intravenous injection, as well as molecular features of the HA protein, such as alterations to the proteolytic cleavage site (WOAH, 2019).

3. Organization of the genome and AIV Structure:

The virion can take on a variety of shapes, including spherical, pleomorphic, and filamentous ones. A single virion's diameter typically falls between 80 and 120 nm, however filamentous forms can reach several hundred nm, according to Cox et al. (2000). This encapsulated virus has a helical nucleocapsid (Lamb and Krug,

2001). The genome of the avian influenza virus is a negative single-stranded RNA molecule with a length of about 13.5 kilobases. This genome consists of eight distinct segments, each of which codes for a unique protein required for the completion of the viral life cycle. These proteins are polymerase basic 2 (PB2, 759 amino acids), polymerase basic 1 (PB1, 757 amino acids), , polymerase acidic (PA, 716 amino acids), hemagglutinin (568 amino acids), nucleoprotein (NP, 498 amino acids), neuraminidase (499 amino acids), matrix protein 1 (M1, 252 amino acids), matrix protein 2 (M2, 97 amino acids), NS1 (225 amino acids) &NS2 (121 amino acids) (Sangsiriwut et al., 2018; Noor et al., 2022).

In the mature virion, there are two types of proteins; 1) Structural proteins which comprise surface proteins, including, hemagglutinin, membrane ion channel proteins, neuraminidase, & internal proteins, encompassing nucleoprotein, matrix protein, & the polymerase complex, composed of polymerase basic protein two, polymerase basic protein one, & polymerase acidic protein; 2) Non-structural proteins encompass non-structural protein one as well as non-structural protein two, which are recognized as the nuclear export protein and mostly detected in host cells, while some are also present in the virion (Swayne, 2008).

3.1 Influenza hemagglutinin:

HA, a glycoprotein with a molecular weight of 165,447.31 Dalton, is present on the viral envelope. HA's triple chains (L, J, and H) make up polymer 1, which has 328 amino acids, while polymer 2 has three chains (M, K, and I), which have 160 amino acids. According to Gamblin et al. (2004), the computed extinction coefficient is reported as 270,060, while the theoretical isoelectric point (pI) is indicated as 6.15.

The hemagglutinin monomer has four antigenic sites: site A, which is located 8 Å below the molecule's surface; site B, which binds to specific areas of the α-helix externally to form a little pocket or area where sialic acid can adhere. About 60 Å separates site C from the molecule's bottom. Site D uses two beta-sheets stacked in a jelly roll-like configuration. The virulent and antigenic properties of IAV can be changed by any mutation within these antigenic regions (Schweiger et al., 2002). HA is a trimeric glycoprotein made up of hemagglutinin 1 & HA2, two polypeptide chains. The globular head region of the hemagglutinin monomer is mainly made up of HA1, & it is joined to a fibrous stalk domain formed of the polypeptide segments hemagglutinin 1 in addition to HA2. The receptor binding domain (RBD), antigenic regions, N-linked glycosylated carbohydrate (GS), proteolytic cleavage site (PCS), and immunogenic epitopes are among the important structural elements of the hemagglutinin 1 protein (Chen et al., 1998; Steinhauer, 1999; Brown, 2000). Whereas the transmembrane domain & fusion peptide are connected to the HA2 protein (Armstrong et al., 2000). The receptor-binding pocket of the AIV typically attaches to α two-three linkage sialosides, which are widely distributed in the avian digestive tract. However, viruses that have adapted to humans exhibit selectivity for the α two-six linkage, mainly in the respiratory system (Parrish & Kawaoka, 2005). After first being produced as a polypeptide chain including the encoded domains HA1 in addition to HA2, HA is eventually translocated into the endoplasmic reticulum & ends up on the surface of cells (Klenk et al., 1975).

3.2 NA protein:

NA structural characteristics include a square box-shaped head & a stalk on the virus envelope's surface. It can identify sialic acid (Itzstein et al., 1993). The single polypeptide chain A with 4 domains and a molecular weight of 46502.45 Dalton is revealed by the 3D structure of NA at 2.2 Å (Varghese and Colman, 1991). Also, according to Varghese and Colman (1991), it has an extinction coefficient of 85005 and a predicted pI of 6.48. NA tetramers of identical polypeptides occupy twenty to thirty percent of the glycoproteins present the virion's surface. For example, forty to fifty NA spikes in addition to 300–400 HA spikes are often found in a virion measuring 120 nm (Ward et al., 1983; Varghese et al., 1983; Moules et al., 2010).Together, the 4 monomers, which have a combined length of about 470 AA, form distinct structural components including the stalk, catalytic head, transmembrane region, and cytoplasmic tail (Harris et al., 2006).

Blok and Air (1982) reported that the N-terminal region of the cytoplasmic tail of NA exhibits nearly 100% sequence conservation across all IAV subtypes and is strongly involved in fundamental viral activities. According to Blok & Air (1982) also Air (2012), the transmembrane region, which joins NA to the viral envelope, is expected for formation of an alpha helix and include a diverse range of amino acids. Viruses move from the endoplasmic reticulum to the apical surface based on signals produced from transmembrane domains which also aid in viruses' attachment to lipid rafts (Barman and Nayak, 2000).

The NA domain stalk seen in several influenza subtypes of a virus may share structural characteristics. The amount and sequences of amino acid residues, however, might vary significantly (Blok & Air, 1982). Despite this variation, it is crucial to remember that every NA stalk domain has a few structural traits in common, like having at least one cysteine residue and a possible location for glycosylation (Blok and Air, 1982; Air, 2012).

All NAs have a catalytic head that is made up of four monomers arranged in a box shape. Every monomer has the appearance of a propeller structure with six blades, each of which has four anti-parallel β-sheets. Disulfide bonds hold these β-sheets together, and loops of different lengths connect them (Varghese et al., 1983). On the surface of every monomer is a functioning catalytic site that is oriented horizontally as opposed to vertically. This keeps the virus from entangling itself by enabling it to break sialic acids from adjacent membrane glycoproteins (Colman et al., 1983; Burmeister et al., 1992).

Three groups comprise the nine classical subtypes of NA: 1st group consists of N8, N5, N1, and N4, 2nd group consists of N9, N7, N6, N2, and N3, and 3rd group contains NA of influenza B viruses. Two new NA subtypes, N11 and N10, have been originate in bats recently (Tong et al., 2013).

The NA tetramer can be seen on the virion surface as solitary spikes or in small clusters surrounded by HA, according to cryoelectron tomography. The neuraminidase may protrude from the viral envelope depending on the length of the stalk area in comparison to the HA, which could have an influence on the total amount of activity of the enzyme (Harris et al., 2006; Matsuoka et al., 2009).

3.3 Matrix (M) protein:

The M gene produces membrane proteins in addition to matrix proteins such as M1 and M2. Segment 7 of the AIV is responsible for translating these M proteins, and the M gene has 1027 base. Interestingly, M2 is found between nucleotide locations 26 to 51 and 740 to 1007, whereas M1 is found between places 26 to 784 (Lamb et al., 1981).

3.3.1 Matrix 1 protein:

The exposure of crystalline structure of influenza M1 to X-rays at certain conditions displayed that N-terminal region (2-158 amino acid) showed a dimeric structure with a positive charge on its hydrophobic surface (Harris et al., 2001). The M1 protein links membrane proteins to the internal core of the virus. L domain motif of M1 is proposed to be essential component for the assembly & release (budding) of the virus (Nayak et al., 2004).

3.3.2 Matrix 2 protein:

The Matrix 2 protein consisting of 97 amino acids (Zebedee and Lamb, 1989) has important sites for drug resistance, e.g., adamantine, especially at positions 26, 27, 30, 31, and 34 amino acids (Liu et al., 2010). The surface of the infected cell expresses M2 which is incorporated in the virus on completion of its replication. According to Abbas and Abidin (2013), it is significant because it facilitates virus replication at lower doses.

3.4 Polymerase gene complex (1, 2 and PA proteins):

Three subunits make up the influenza virus polymerase complex: PA (polymerase acidic protein), PB1 and PB2 (polymerase basic protein-1 and polymerase basic protein-2). When discussing influenza C, PA is referred to as P3. The eight viral ribonucleoprotein (vRNP) segments containing one polymerase heterotrimer, are present in both influenza A and B virions (Moeller et al., 2012).

PB1 is situated on the IAV's mRNA second segment and comprises two ORFs: PB1-F2 and PB1. These proteins are located within the cellular mitochondria of eukaryotic cells, where they contribute to the intracellular localization of PB1. Furthermore, the mRNA second segment is used to synthesize another polypeptide, PB1 N40 (Wise et al., 2009). PB1-F2 is composed of 90 amino acids and is present in the cytosol, nucleus, and especially the mitochondria of afflicted cells. According to research, PB1-F2 contributes to the development of protein channels in the mitochondrial membrane; this activity is especially important for the C-terminal region (Henkel et al., 2010).

The MW of the PB1-PA complex is 63,530.73 Da. The two polymer chains that make up the PB1-PA complex are the RNA-directed RNA polymerase catalytic subunit polypeptide chain B, which has a size of 81 aa, together with the polypeptide L chain A of the acidic protein polymerase, which has 478 AA. (Obayashi et al., 2008).

3.5 Nucleoprotein:

The molecular weight of the NP is 170419.81 Dalton. It is placed in the category of viral nuclear proteins. It is a polypeptide nucleocapsid protein made up of 3 chains: A, B, & C in a single polymer. With an extinction coefficient of 167175 and a theoretical pI of 9.24, the total size of nucleoprotein is 499 AA. It appears as a crescent-like form with a body, head, in addition to flexible tail loop for attachment, according to the crystalline structure at 3.2 Å (Tarus et al., 2012).

Located outside of the NP oligomer, in the center of the head and body, lies the RNA binding groove. The NP body domain contains a viral polymerase binding site. Additionally, Ye et al. (2006) recognize the tail loop as a potential target for the development of antivirals. After the eight RNA segments are wound around NP subunits, vRNP is produced. According to Hutchinson et al. (2009), NP is a basic protein that is positively charged and located in segment 5 of the virus genome. NP is necessary for the virus to replicate (Huang et al., 1990).

3.6 Non-Structural (NS) proteins:

RNA segment 8 is used to create NS1 and NS2. The reason they are classified as non-structural proteins is that they are produced in impacted cells independently of the virion.

3.6.1 NS1

NS1 is made up of three different domains and has an MW of about 26 kDa. It is composed of a C-terminal tail, an effector domain (ED) in the middle, and an N-terminal RNA-binding domain (RBD). The multifunctional properties of NS1 are a result of the interactions between each of these domains and particular cellular components (Kawaoka, 2006; Tscherne and Garcia-Sastre, 2011).

NS1 normally consists of 230 AA, although this length can vary due to deletions in the linker region or changes in the placements of stop codons within the disordered C-terminal end (CTE) of the ED (Abdelwhab et al., 2016b). NS1 is made up of (ED, residues 88–230) and (RBD, residues 1-73), which are joined by a linker (residues 74–87). The most well-known feature of this multifunctional protein is its capacity to inhibit the interferon response. This can happen when the ED domain interacts with different cellular proteins or when the RBD binds to RNA-sensors (Marc, 2014).

3.6.2 NS2

It is transcribed from the NS gene via mRNA splicing and consists of 121 AA. It has been found that NS2 interacts with M1 and can exist in pure viruses. The nuclear export of viral RNPs is enhanced by NS2 and M1 (Lommer & Luo, 2002; Iwatsuki-Horimoto et al., 2004;). All IAV strains that have been sequenced share a high level of conservation in NS2. An amphipathic C-terminal domain with two α-helices is a part of the NS2 structure, while an N-terminal domain with two nuclear export signals is also present (Akarsu et al., 2003).

4. Viral stability:

4.1 Susceptibility to disinfectants

AIVs demonstrate a general vulnerability to various disinfectants, such as sodium hypochlorite (200 ppm), benzalkonium chloride (1,000 ppm), iodophor as I2 (75 ppm), 0.12% ortho-phenylphenol, and 0.02% glutaraldehyde solutions. This results in the inactivation of the virus within a span of 10 minutes (Block, 2001). Other effective disinfectants include a 1:16 dilution of PREvail™ (accelerated hydrogen peroxide), Vesphene®IIIse (phenol), VERT2GO SABER Concentrated (hydrogen peroxide), NEUTRAQUAT 256, and Quat-3 (quaternary ammonium compounds) (CFIA, 2022).

4.2 Physical inactivation

Applying heat through incubation at 56-60°C for 60 minutes proves effective in inactivating various H5, H7, as well as H9 subtypes (PHAC, 2023). Incubating the virus in low (1-3) or high (10-14) pH solutions is reportedly successful in inactivating IAV subtypes H5, H7, & H9, although the virus's suspension medium may influence the effectiveness of pH on virus infectivity (PHAC, 2023). H7N9 strains lose their infectivity following heat treatments at fifty-six °C for thirty minutes, sixty-five °C for ten minutes, seventy °C, 75°C, & 100°C for one minute (Zou et al., 2013).

4.4 Survival outside the host:

The HPAI H5N1 exhibits survival times up to eighteen hours at 42 °C, a whole day at 37 °C, five days at 24 °C, as well as eight weeks at four °C in both dry as well as moist poultry feces (Kurmi et al., 2013). In chickens experimentally infected, H5N1 can endure up to 160 days in muscle, 240 days in feather tissues, & 3 days in the liver at 4°C. At 20°C, the virus remains viable for up to thirty days in feather tissues, twenty days in muscle, and three days in the liver (Yamamoto et al., 2017). The survival time for H5N1 is approximately 26 hours on plastic surfaces and about 4.5 hours on human skin surfaces. In contrast, subtypes H5N3, H5N9, and H7N9 are rendered inactive on plastic surfaces within 10 hours and within 1.5 hours on human skin (Bandou et al., 2022).

5. Virus replication:

The virus initially interacts with the host cell through the nine-carbon acidic monosaccharide known as N-acetyl neuraminic acid, or sialic acid (Couceiro et al., 1993). The frequent sialic acid connections are 2, 3, and 2, 6, and influenza viruses have a significant affinity for them, according to Wang et al. (2013). A virus first produces the HA0 precursor of the HA glycoprotein in the host cell, which host serine proteases cleave into subunits (HA1 and HA2), resulting in infectious viral particles (Klenk and Garten 1994). The virus enters the host cell by means of receptor-mediated endocytosis, which occurs at the inner face of the plasma membrane and produces endosome formation (Rust et al., 2004; Dou et al., 2018). Through a conformational shift of HA0 brought by this process, the M2 ion channel is opened, allowing the viral and endosomal membranes to join. Because of this, the virion interior becomes acidic throughout the fusion process, releasing the viral ribonucleoprotein (vRNP) from M1 and allowing it to enter the host cell's cytoplasm (Pinto and lamb, 2006; Pielak and Chou, 2011). Through a process called "cap snatching," the mRNA obtains a 5' capped primer by the PB2 protein which extracts the primer from the host mRNA (Al-Mubarak, 2014). Following that, nuclear pores allow passage of the ensuing positive-sense viral mRNA to ribosomes for translation (Swayne and Pantin-Jackwood 2008). In the host cell's cytoplasm, a wide range of proteins are produced, including M1, NP, PA, and polymerase basic (PB1 and PB2), nonstructural (one & two), in addition to NP. Following their transfer to the nucleus, these proteins are involved in a multitude of processes including transcription, splicing, and

replication of matrix & nonstructural proteins and others (Staller et al., 2021). In the nucleus, freshly synthesized PA, NP, PB1, PB2, as well as NS2 proteins form vRNP complexes. M1 proteins speed up vRNP trafficking to the cytoplasm after the formation of the M1-vRNP complex. The nuclear export signal (NES) regulates the transport of vRNA complexes into the nucleus and is carried by NP proteins and blocked by M1 proteins (Garcia-Moreno et al., 2018). The budding and release mechanisms of newly synthesized viruses at the apical plasma membrane complete viral multiplication. M1 peptide accumulation on the cytoplasmic side of the lipid bilayer is most likely the cause of the budding process, which involves interactions between M1 complexes and the cytoplasmic terminals of envelope proteins (M2, HA, and NA proteins) (Nachbagauer and Palese, 2020). Before the developing virion leaves plasma membrane, the sialic acid residues in plasma membrane are cleaved by NA, which expedites the release of virus particles into the extracellular medium (McAuley et al., 2019).

6. Pathogenesis:

The virulence of avian influenza viruses in domestic fowl determines their classification as either HPAI or LPAI (WOAH, 2018). While LPAI infections are usually milder in all bird species, HPAI viruses usually cause serious sickness in flocks of chickens, turkeys, and waterfowls (WOAH, 2023a).

6.1 LPAI:

Due to its restricted ability to multiply in the small intestine and tracheal tissues, it is characterized by a low mortality rate & limited capacity to cause a disease to chickens (Franca and Brown, 2014; CDC, 2017; Nuñez and Ross,2019). The low pathogenicity H5/H7 subtypes, which are commonly found in poultry & wild waterfowl, can undergo mutations in the HA proteolytic cleavage site due to insertion and also recombination mechanisms. This could potentially cause the emergence of HPAI viruses (Rabadan and Robins, 2007; Lee et al., 2021)

6.2 HPAI

The HPAI viruses can enter birds' bodies through the respiratory and intestinal barriers, travel to the blood, & impact several organs (WOAH, 2023a). Interferons (IFN), antiviral cytokines, and cytokines all flood into infected chickens to stop the virus from replicating. Nonetheless, certain cytokines that are stimulated, such as IL-6, IFN, TNF-α, & IL-8 can be involved in the harmful impact of influenza (Kuchipudi et al., 2014). Birds' respiratory and digestive systems are the sites of HPAIV H5 virus replication (Jeong et al., 2009; Burggraaf et al., 2014). Sneezing, nasal discharge, diarrhea, coughing, body part discoloration, appetite loss, low energy, swelling, malformed eggs, and coordination problems are only a few of the clinical symptoms of infection (Franca and Brown, 2014; CDC, 2017). HPAI viruses, of which subtypes H5 & H7 are known to be a reason for acute also severe diseases in a variety of economically relevant birds (WOAH, 2023a).The main steps of HPAI viruses pathogenesis are summarized in **Figure 1**.

6.3 Role of AIV proteins in virus pathogenesis and replication:

6.3.1 HA protein:

HA protein attaches to sialic acid on the surface of host cells to facilitate the virus's entry into the cells. The lower respiratory tract of humans & the gut and digestive tract of avian species are rich in sialic acids α-2,3-gal, which are favorite by strains that have adapted to life in birds (Matrosovich et al., 1997; Costa et al., 2012).

The HA0 is one of the progeny viruses that are produced once the virus enters the cell. The functional components HA1 and HA2 are created when the host cellular proteases cleave HA0 at the proteolytic cleavage site. The creation of infectious viruses depends on this cleavage. A conserved glycine (G) is usually next to an arginine (R) residue in the cleavage site area of HA0 (Garten & Klenk, 1983; Swayne et al., 2020). The quantity of basic amino acids located at the HA cleavage site has a significant impact on the virulence of IAV. According to Bosch et al. (1981), Kawaoka et al. (1987), Horimoto & Kawaoka (1994), and Swayne et al. (2020), this factor determines which proteases cleave the HA0, hence determining the host's tissues and cell types where viral replication occurs. It is possible to classify the cleavage site of IAV's HA as monobasic or dibasic, e.g., PEKQTR/GLF or multibasic (e.g., PQRKKR/GLF). 1 or 2 nonconsecutive basic amino acids, like arginine (R) or lysine (K), are typically present at critical locations within the mono- or dibasic cleavage site. Trypsin or trypsin-like proteases can cleave this location, which restricts the virus's ability to replicate inside the epithelial cells lining the digestive & respiratory systems (Suarez, 2016; Swayne et al., 2020). A rise in the amount of basic amino acids or an extension of the proteolytic cleavage site to at least four basic amino acids make the virus extremely dangerous. This alteration permits a variety of widely distributed cellular proteases, for example Furin-like proteases, to cleave HA0. As a result, the virus becomes more capable of replicating in cells across many organs, increasing its likelihood to induce systemic illness and fatal infection in gallinaceous hosts (Swayne et al., 2020). According to Bogs et al. (2010), the polybasic HA cleavage site is required for the promotion of viral neurotropism, which indicates the virus's inclination for infecting nerve tissues.

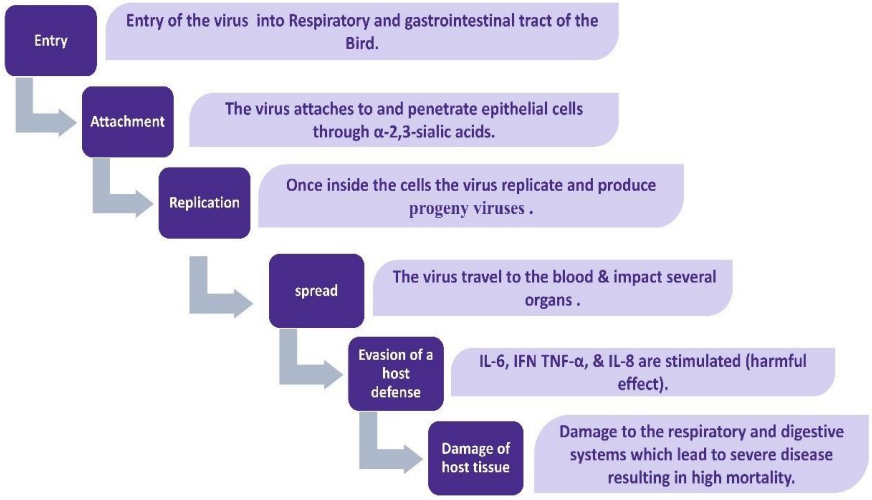

Figure 1. Overview for pathogenesis of highly pathogenic avian influenza virus in birds

6.3.2 NA protein:

Sialic acid, a terminal structure present on cell surfaces, interacts with both HA and NA. Later on in the infection process, NA enzymatically eliminates sialic acids from newly synthesized HA and NA on developing virions as well as on cellular receptors. Therefore, it prevents virions from aggregation, and makes viral progeny to be released efficiently (Palese et al., 1974). Because NA's sialidase activity breaks down sialic acids on decoy receptors such mucins, it helps the virus enter cells. It is thought that this step is essential for the entrance of a virus. NA activity's role in this stage of the entrance process is supported by experiments conducted with medications that inhibit NA activity (Matrosovich et al., 2004; Ohuchi et al., 2006; Su et al., 2009). Pathogenicity is increased by NA's lack of the stalk region, which is shown in HPAIVs. Nonetheless, according to Stech et al. (2015), restoring the NA stalk area reduces pathogenicity in hens. Compared to wild-type viruses, which often have less lethality, deletion of the stalk region increases lethality & transmission. Moreover, according to Sorrell et al. (2010), this deletion not only impacts H5 viruses but also changes the tropism of the H2N2 virus in hens from the intestinal to the respiratory tract.

6.3.3 Matrix protein:

6.3.3.1 M1

M1 is regarded as a versatile-function protein that contributes to many phases of the viral life cycle. Infected cells have it in both the cytosol & the nucleus. A nuclear localization signal (NLS) has been found in M1, indicating that it is imported into the nucleus to aid in the nuclear transfer of freshly formed RNPs (Martin and Helenius,1991; Bui et al., 1996; Brunotte et al., 2014).

M1 is thought to undergo a conformational change, or rearrangement, in its polymer structure when it is exposed to low pH during viral entry. In the end, this phenomenon makes it easier for M1 to separate and disassemble across the membrane (Calder et al., 2010; Fontana and Steven ,2013; Watanabe et al., 2014; Brunotte et al., 2014). M1 departure from vRNPs is facilitated by its interactions with host cell proteins, particularly (TNPO1) the nuclear transport factor transportin-1 (Miyake et al., 2019).

6.3.3.2 M2

Protons can enter the virion interior through the M2 channel, which lessens the connection between M1 and viral vRNPs in the viral core. Moreover, potassium ions are conducted into the virion M2 in late endosomes with an acidic environment (pH 5.4–6.0), which disrupts vRNP-vRNP connections (Pinto et al., 1992; Stauffer et al., 2014). M2 is necessary for the assembly and budding of viruses. At the distal end of the membrane, the M2 cytoplasmic part interacts with M1 to facilitate virion assembly (Chen et al., 2008). To regulate the budding process, M2's membrane-proximal cytoplasmic amphipathic α-helix is crucial. It builds up at the edge of neuraminidase and HA-containing lipid rafts on the plasma membrane of infected cells, changing the curvature of the membrane. As a result, freshly formed virions are eventually released (Rossman et al., 2010).

Several host cell activities are changed by M2. Its conserved LC3-interacting motif near the carboxy terminal end of its cytoplasmic domain interferes with autophagy, and its ion channel activity can activate inflammasomes in myeloid cells (Ichinohe et al., 2010; Beale et al., 2014).

6.3.4 Polymerase gene complex:

According to Khiabanian et al. (2009), PB1 is involved in reassortment events both within and between hosts. In addition, PB1 possesses RNA-dependent RNA polymerase (RdRp) motifs, complementary RNA, viral RNA, and a conserved nucleotide binding domain. Thus, adaptation and reassortment may be linked to the viral polymerase genes (Li et al., 2009). Viral RdRp, which is made up of a triple complex in the nucleus that is made up of the three subunits PA, PB1, and PB2, catalyzes complex processes that the vRNA goes through (Deng et al., 2005).

Through its N-terminal domain, PB2 plays a crucial role in the transcription of the virus and functions as a cap-binding protein (Gastaminza et al., 2003). The precise method by which PB2 suppresses interferon-β expression is yet unknown, despite its known effect. Furthermore, according to Graef et al. (2010), PB2 is essential to the virulence of the influenza virus. It is a crucial component of the virus's polymerase complex and is required for the virus to replicate. Furthermore, as research involving pigs, squirrel monkeys, mammalian cells, and mice have shown, PB2 is important in determining pathogenicity and host range (Manzoor et al., 2009).

Since PA is a phosphoprotein and has a third amino-terminal domain located close to its nuclear localization signals, it functions as a protease. Reduced polymerase activity brought on by PA mutations can lower the amount of cRNA that is produced from vRNA (Abbas and Abidin, 2013).

6.3.5 Non-structural protein

6.3.5.1 NS1

The Non-structural protein 1 has multiple roles in disease pathogenesis, virulence, and host-pathogen relationships. It consists of two domains: the ED and the RBD, sometimes referred to as the N-terminal structural domain. The RBD inhibits the production of IFNα/β and helps shield the virus from the host immune response by limiting the function of cellular antiviral proteins, particularly the 2'-5'-oligoadenylate synthetase (OAS)/RNase L pathway (Krug et al., 2003). The effector domain inhibits the host antiviral response at the cellular mRNA level by preventing mRNA attachment and removal and interfering with the polyadenylation specificity factor. The function of the poly(A)-binding protein (PAB II) is thereby inhibited (Wang et al., 2002). As the virus replicates, NS1 blocks IFN to evade the host immune response. It does this by preventing the manufacture of IFN-inducible antiviral proteins, for example PKR (double-strand break-dependent protein kinase R) as well as 2-5-oligoadenylate synthetase. To induce 2´-5´OAS and PKR, double-stranded RNA is required (dsRNA). NS1 prevents PKR activation and cellular 2-5 OAS activity by physically attaching to dsRNA (Hale et al., 2006). Pre-mRNA splicing and the suppression of poly A mRNA export in nuclear post-transcriptional processes are two processes in which NS1 is engaged. Moreover, NS1 influences the translation of viral mRNA in the cytoplasm by binding to viral RNA, which in turn influences the phosphorylation of eukaryotic translation initiation factor 2α. Notably, NS1 has a leucine-rich hydrophobic spacing & a nuclear export signal (NES) that are critical to its function. Furthermore, when leucine is exchanged for alanine, its nuclear export function is compromised (Yongzhong et al., 1998).

6.3.5.2 NS2

Because NS2 modifies the amounts of RNA, there is a corresponding increase in viral replication and a decrease in the accumulation of transcription products. NS2 can effectively regulate the transcription and translation of viruses due to its dual role (Robb et al., 2009).

6.5 Assessment of pathogenicity

To et al. (2013) state that HPAI is indicated by intravenous pathogenicity index (IVPI) values greater than 1.2 or a mortality rate during the 10-day period that equals or exceeds 75% of the total poultry population. HPAI strains typically belong to the H5 and H7 subtypes, and they cause more than 90–100% of bird deaths within 48 hours of the sickness beginning (To et al., 2013; Kanaujia et al., 2022). Also, sequencing analysis of HA cleavage sites has been used for assessing AIV pathogenicity (Swayne et al., 2020). As explained previously in the section of role of HA protein in pathogenesis, multibasic amino acids cleavage site indicates HPAI while mono- or dibasic amino acids cleavage site indicates LPAI.

7. The mechanism and the risk of AIV evolution

The continuous change of influenza viruses is primarily driven by three mechanisms: antigenic shift (re-assortment), antigenic drift (mutation), and, in rare cases, recombination. Gene fragments of the influenza virus can occasionally spread to other animals, even though distinct virus lineages are typically restricted to specific hosts. These interactions may lead to pandemics affecting people, smaller animals, and birds (Webster et al., 1992).

7.1 Antigenic drift

The influenza virus's RNA polymerase is known to be incapable of proofreading. As a result, during viral replication, erroneous nucleotide integration frequently occurs at a rate of 10^{-3} to 10^{-4}. This high rate of mutation is primarily responsible for the genetic diversity of influenza viruses (Ahlquist, 2002; Chen and Holmes, 2006). Antigenic drift refers to the process of notable changes occurring in antigenic areas because of continuous point mutations in the influenza virus. These changes cause the antigenic properties of the virus to progressively change, ultimately leading to the emergence of influenza virus with new antigenic characteristics. The virus poses obstacles to immunity because of its ability to drift its antigens and evade the population's immunological defenses (Carrat and Flahault, 2007). Immune evasion may occur when alterations occur in the HA and/or NA IAV proteins. Even slight changes to these surface proteins, whether from vaccination or prior infections, can neutralize the host's developed immune response to the invasive virus. This makes it more difficult for the immune system to recognize the altered viral variants, which hinders the interaction between the antigen and the antibody.

Moreover, the amino acid alterations in the HA protein have the power to alter the influenza virus's preferred receptor. Research has indicated that mutations like G186V in the HA protein could help avian H7 viruses adapt to human-type receptors (Xiong et al., 2013, Dortmans et al., 2013). The HPAI viruses HA cleavage site alterations are caused by a number of mechanisms: (a) When non-basic amino acids are replaced with basic ones, a protective glycosylation site may occasionally be removed; (b) several basic amino acids may be added from duplicated codons; (c) brief additions of both basic and non-basic amino acids from an unknown source; or (d) nonhomologous recombination occurs when RNAs from viruses or cells, such as host 28S RNA or RNA coding M or NP protein, and results in the extension of the proteolytic cleavage site. HPAI viruses H5/H7 have been reported to have modifications (a), (b), and (c), but only H7 HPAI viruses have been identified to have variation (d) (Suarez et al. 2004 ;Pasick et al. 2005; Maurer-Stroh et al. 2013; Swayne et al. 2020). Higher binding affinities of the A/Anhui/1/13 (H7N9) virus to α-2,3 and α-2,6 sialic acid receptors were the outcome of both the K58I substitution and the G219S mutation in the HA protein (Schrauwen et al., 2016). Furthermore, data indicates that the PB2 protein's E627K mutation promotes the reproduction of influenza viruses harboring PB2 from avian sources in human respiratory epithelial cells (Mehle and Doudna 2009). The duck 3286/H7N9 virus's PB2 gene mutation has been shown to increase polymerase activity and encourage viral replication in human cells (Li et al., 2017b). The evidence suggests that the PB2 polymerase protein's E627K mutation is a major factor in avian influenza viruses' host range (Arai et al., 2016).

7.2 Antigenic shift

Re-assortment is essential for creating "novel" influenza virus strains since the IAV genome is segmented (Vergaraalert et al., 2014). In 1996, geese in the Guangdong province (Gs/GD) were found to harbor the H5N1 subtype of the HPAI virus (Lee et al., 2017b). The H5N8 virus underwent genetic evolution after first emerging in chicken epidemics in 2013, giving rise to a range of HA genetic progeny. Subtype H5N8 clades 2.3.4.4 Gs/GD HPAIV were then created by reconstructing it utilizing additional internal and NA genes (Zhao et al., 2012). After then, there was an outbreak in South Korea in January 2014 (Lee et al., 2014). Around the end of 2014, the HPAI H5N8 virus spread over various European countries (El-Shesheny et al., 2017). During summer 2016 sampling, a novel strain of H5N8 was discovered in wild aquatic birds in western Siberia (Lee et al., 2017b). The H5N8 virus strain killed three species of wild migrating birds at Qinghai Lake, China, in May 2016. Genetic analysis indicates that the distinct re-assortant virus is a member of the group B H5N8 viruses, and reassortment events most likely occurred in early 2016. Therefore, as wild birds migrated, the H5N8 virus may have travelled to new areas along their flight paths (Li et al., 2017a). Moreover, reassortment between the Gs/GD lineage H5N8 virus and viruses originating in North America led to the emergence of the H5N1 and H5N2 viruses in the US (Jhung and Nelson, 2015). A novel strain of the HPAI clade 2.3.4.4 virus (H5N2) caused an outbreak in British Columbian chicken farms in November 2014. Later, raptors, backyard poultry containing domestic ducks & geese, and wild waterfowl were found to harbor it in the United States (Wu et al., 2015; Pantin-Jackwood et al., 2017).

7.3 Recombination:

Natural selection of IAV uses genetic diversity as its substrate, and genetic recombination is essential to the creation of genetic diversity. Two main mechanisms are involved in the recombination process of IAVs. Non-homologous recombination is one such method that is typified by the genetic material being exchanged across dissimilar RNA pieces (Orlich et al., 1994; Suarez et al., 2004). Although its frequency is disputed and frequently regarded as small, homologous recombination is another mechanism of recombination in IAVs. Genetic exchange is thought to happen when the viral polymerase switches templates during RNA replication. However, during AIV replication, the quick packaging of genomic RNA with ribonucleoprotein aids in preventing template-switching, which adds to the impression that homologous recombination in influenza viruses is uncommon (Chare et al., 2003). Nonetheless, it has been indicated that homologous recombination may occur during the replication of the AIV. According to these findings, recombination between genetically distinct variants has produced novel genotypes and may have an impact on the evolution of H5N1 viruses. This highlights the dynamic aspects of viral evolution and refutes the widely held belief that homologous recombination in influenza viruses is uncommon (Rubio et al., 2013). It has been suggested the novel H7N9 virus PB1 region as the site of recombination. In particular, a segment from HPAIV strain A/tree sparrow/Thailand/VSMU-16-RBR/2005 (H5N1) appears to have been integrated into its PB1 segment between nucleotide locations 490 and 780. According to Chen et al. (2016), this recombinant PB1 region most likely first appeared in H9N2 or H5N1 viruses in 2007 before being incorporated into the novel H7N9 virus, which sparked an outbreak in 2013.

8. Mixed infection of AIV and other viruses or bacteria and its effect on pathogenicity:

Egypt's commercial chicken flocks have seen a marked rise in respiratory disease outbreaks in recent years. The poultry business has a considerable problem because of the complicated nature of these diseases, which display varied death rates and diverse clinical symptoms (Roussan et al., 2008a). The main causes of these respiratory problems are NDV, IBV, and AIV (both high and low pathogenic strains). According to Radwan et al. (2013), Hassan et al. (2016), Samy & Naguib (2018), these viruses are the main culprits behind respiratory illnesses with significant fatality rates in chicken farms, either on their own or in combination with other viruses or bacterial agents. Some of possible effects of mixed infection of AIVs and other pathgens are listed in **Table 1&2**.

When a host contracts a different virus from the common one, viral interference may occur (Dianzani, 1975). Viral interference is the term for when virus-infected cells prevent the growth of other viruses, whether they are of the same or different type (Dianzani, 1975; DaPalma et al., 2010). Viral interference can be explained by several processes, including: (1) competition for cell receptor

attachment during replication; (2) intracellular competition involving host machinery; and (3) interference caused by interferon that is generated by the virus. Observable variations linked to mixed virus infection include modifications in tissue tropism, viral replication patterns, immune responses, and pathological responses (DaPalma et al., 2010).

There have been several reports of co-infections between the HPAIV and the LPAIV in natural cases from several nations. Examples include China, Bangladesh, and Egypt where HPAIV H5N1 and LPAIV H9N2 were co-infected (Arafa et al., 2012). Based on experimental research, it appears that LPAIV H9N2, such as A/Chicken/HK/G9/97, can shield hens from the deadly HPAIV H5N1 virus (Seo & Webster, 2001). Remarkably, co-infected chickens tend to excrete a large amount of H5N1 in their faeces and a small amount in their trachea. These co-infected chickens usually exhibit modest clinical indications, such as sneezing, nasal discharge, and ruffled feathers. According to Khalenkov et al. (2009), cross-reactive cellular immunity brought on by H9N2 influenza viruses is thought to represent the protective mechanism against HPAIV H5N1.

As a gamma-Corona virus, the infectious bronchitis virus (IBV) is frequently found in hens co-infected with AIVs, which causes the poultry sector to suffer large financial losses (Hassan et al., 2016). Potential effects of co-infection with IBV include a reduction in the stability of the HA of H9N2 AIV in afflicted flocks (AboElkhair et al., 2021). According to Haghighat-Jahromi et al. (2008), in experimental settings, coinfection with the H9N2 virus and the IBV live vaccination prolonged the H9N2 virus's shedding duration, worsened clinical symptoms, increased mortality rates, and caused macroscopic lesions in embryos.

Although the symptoms of HPAI and vNDV are similar and difficult to differentiate clinically, spontaneous co-infections with both strains of the virus have been reported in numerous countries (Akaike et al., 1989). Velogenic NDV hampered HPAIV replication in an experimental co-infection paradigm, which decreased the number of birds shedding HPAIV. In spite of this, all birds died from the infection between 1.9 and 5.2 days, and at high doses of vNDV, there was no discernible difference in clinical symptoms between the single-infected and co-infected groups. Remarkably, co-infected chickens' survival was enhanced by a low dose of vNDV (Costa-Hurtado et al., 2015). Conversely, hens exposed to a lower concentration of HPAIV prior to infection with the milder mesogenic strain of NDV demonstrated a drop in HPAIV replication and an increase in survival rates. In conclusion, variables including the virulence, concentration, and time of infection for the viruses implicated affect the likelihood of viral interference (Costa-Hurtado et al., 2015).

In two spontaneous cases, co-infections of the vNDV with H5N8 were found in one of the duck farms in Egypt, while co-infections with H5N1, H5N8, and H9N2 were found in the other (Hassan et al., 2021). Furthermore, it was shown that the vNDV and the AIV interfered with one another in co-infected ducks (Pantin-Jackwood et al., 2015).

Compared to ducks infected with vNDV alone, those infected with LPAIV shed less vNDV but did not exhibit any symptoms. Nevertheless, there was no discernible impact on LPAIV shedding. Ducks that were infected with vNDV alone had a higher survival rate than those who were infected with vNDV and then HPAI. Additionally, there was a decrease in the spread of vNDV to interacting ducks, suggesting a conflict between the viruses' pathogenesis and transmission (Pantin-Jackwood et al., 2015).

Waterfowl have been found to be infected with both NDV and LPAIV. As a result of AIV surveillance in the United States, multiple NDV isolations have been reported (Rosenberger et al., 1974; Slemons and Easterday, 1976; Smitka and Maassab, 1981; Deibel et al., 1985; Hinshaw et al., 1985; Coffee et al., 2010; Goekjian et al., 2011; El Zowalaty et al., 2011).

In cloaca swabs of one-month-old mallards, the experimental study discovered that co-infection with lentogenic NDV and LPAIV led to decreased lentogenic NDV shedding but increased LPAIV shedding. This implies that both viruses may have adapted to live in common waterfowl, which may have the effect of stabilizing their replication and spread throughout wild duck populations (Franca et al., 2014).

Although the specific mechanism is yet unknown, the process of AIV proteolytic activation is well documented when AIV is combined with bacterial infections. The significance of bacterial coinfection is explained by four proposed mechanisms: (1) direct cleavage of the HA; (2) indirect activation of host proteases; (3) functioning as an antagonist to host protease inhibitors; and (4) inducing an increase in host protease release by stimulating the host's inflammatory response (Callan et al., 1997; Akaike et al., 1989; Scheiblauer et al., 1992).

Infections of the respiratory system brought on by viruses can exacerbate secondary bacterial infections in people, animals, and birds. This occurs because of the viruses injuring specific cells, which makes it possible for bacteria to adhere and proliferate (Bakaletz, 1995; El Ahmer et al., 1999). Damage of this kind may alter the immune system's response and impair the body's capacity to eradicate microorganisms (Debets-Ossenkopp et al., 1982; Navarini et al., 2006). It is noteworthy that secondary bacterial infections have an additional effect of increasing the number of bacteria in the body in addition to exacerbating the sickness (Tan et al., 2012). However, bacterial infections in the past may actually aid bird flu viruses, particularly if the bacteria create enzymes that aid in the breakdown of the virus's exterior proteins (Tashiro et al., 1987; Kishida et al., 2004). On the other hand, by increasing the immune system's ability to fight viruses or by stopping the virus from attaching to particular cells, a bacterial infection may lessen the severity of a viral disease (Sid et al., 2016).

When Staphylococcus species and AIVs co-infect, the soluble proteases made by the former can cause the latter to activate the AIVs' HA (Scheiblauer et al., 1992). Relatedly, most strains become at least 100 times more contagious when AIVs are treated in-vitro with Staphylococcus aureus proteases (Tashiro et al., 1987). Furthermore, staphylokinase, which converts chicken plasminogen into plasmin, may indirectly activate the HA (Longping and Whittaker, 2015).

In an experiment, chickens that were first given a mild case of bird flu (H9N2) and then given another dose of E. coli four days later demonstrated significantly higher levels of antibodies against bird flu two weeks later when compared to the chickens that received a second dose of other infections, such as Ornithobacterium rhinotracheale or IBV. Furthermore, the bird flu virus was detected in the pre-infected group up to 14 days after the first infection, whereas it was detected in the group solely infected with H9N2 for 7 days (Bano et al., 2003). Moreover, in an comprehensive experiment exploring the effect of dual infection of H9N2 and avian pathogenic E.coli (O:157), the authors suggested that the long co-infection for one to two weeks may be the real cause for the inflated losses associated with H9N2 infections in commercial broilers (Mahmoud et al., 2022).

9. History and epidemiology of AIV worldwide and in Egypt:

9.1 Host species:

9.1.1 Wild birds:

Because Egypt is strategically located to connect Asia, Europe, and Africa, millions of migratory birds pass through the region every year, especially in the winter. Egypt also has a vast diversity of bird species, both in populations raised by farmers and on intensive farms. Different genotypes that eventually spread to domestic bird populations were produced by evolutionary differences resulting from recombination with different AIV subtypes (Lycett et al., 2020). Most of the variants of AIV are low-pathogenic subtypes, and wild migratory birds serve as a natural reservoir for the virus (Olsen et al., 2006). The diversity of AIV that may be found in wild birds, particularly waterfowl, and their high degree of movement allow for the formation of novel and/or altered

AIV. Bird migration patterns have been connected to the long-distance transmission of HPAI H5N1 viruses (Si et al., 2009). Some AIV subtypes were first discovered in wild birds, whereas other subtypes were first discovered in domestic birds, according to genetic analysis of viruses taken from Egyptian birds. A fisherman in the northern Egyptian district of Damietta took a cloacal swab from a Eurasian green-winged teal that was kept in a cage. This sample proved to be the first source of the HPAI H5N1 virus of clade 2.2 in Egypt. Then, starting in February 2006, domestic birds (as well as people) were shown to harbor genetically similar HPAI H5N1 viruses (Saad et al., 2007). In November 2016, two Eurasian coots, either dead or sick, were found to have the HPAI H5N8 virus of clade 2.3.4.4 in a live bird and fish market in the Damietta region (Selim et al., 2017). While migratory ducks are thought to have contributed to the introduction of HPAI H5N1 and H5N8 viruses into Egypt, there is currently no proof that they were also responsible for the introduction of LPAI H9N2 viruses (Naguib et al., 2019). Clade 2.3.4.4b HPAI H5N1 variations have been the predominant highly pathogenetic subtype seen in domestic and wild birds since late 2020 (Cui et al., 2022). According to Cui et al. (2022), the HPAI H5N1 variants of clade 2.3.4.4b have created a serious negative impact on both wildlife and the poultry sector due to their huge global dispersion. Several AIV subtypes have been connected to the introduction of migratory wild birds into Egypt (Naguib et al., 2019). It was recently discovered and genetically characterized that the HPAI H5N1 subtype of clade 2.3.4.4b originated in Egypt in 2021–2022. (Mosaad et al., 2023) **Figure 2**.

Table 1. Some proposed effects due to mixed infections of AIVs with other viruses

Co-infection of avian influenza virus with other viruses	Proposed effect on pathogenesis	References
HPAIV & LPAIV	LPAIV could shield hens from the deadly HPAIV H5N1 virus	Seo & Webster, 2001
	Cross-reactive cellular immunity brought on by H9N2 could be the protective mechanism against HPAIV H5N1.	Khalenkov et al.,2009
H9N2 & IBV	Reduction in the stability of the HA of H9N2 AIV in afflicted flocks	AboElkhair et al., 2021
	Worsened clinical symptoms, increased mortality rates, and caused macroscopic lesions in embryos.	Haghighat-Jahromi et al.,2008
HPAIV & vNDV (chickens)	vNDV hampered HPAIV replication in an experimental co-infection paradigm, which decreased the number of birds shedding HPAIV & no obvious difference in clinical symptoms between the single-infected and co-infected groups	Costa-Hurtado et al., 2015
HPAIV & vNDV (Ducks)	A conflict between the two viruses' pathogenesis and transmission has been suggested	Pantin-Jackwood et al., 2015
LPAIV & vNDV (Ducks)	Ducks infected with LPAIV shed less vNDV but did not exhibit any symptoms	Pantin-Jackwood et al., 2015
LPAIV & lNDV (Ducks)	Decreased lentogenic NDV shedding but increased LPAIV shedding	Franca et al., 2014

Table 2. Some proposed effects due to mixed infections of AIVs with bacteria

Co-infection of avian influenza virus with other bacteria	Proposed effect on pathogenesis	References
Bacteria and AIV	Bacteria may aid AIV, if the bacteria create enzymes that assist in the breakdown of the virus's exterior proteins	(Tashiro et al., 1987; Kishida et al., 2004)
	may lessen the severity of a viral disease by increasing the immune system's ability or by stopping the virus from attaching to cells.	Sid et al., 2016
Staphylococcus species & AIVs	The soluble bacterial proteases activate the AIVs' HA.	Scheiblauer et al., 1992
	Staphylokinase, which converts chicken plasminogen into plasmin, may indirectly activate the HA.	Longping and Whittaker 2015
H9N2 & E. coli	E. coli worsens the clinical condition of the birds formerly infected with H9N2 virus	Bano et al., 2003
	Long dual infection of H9N2 and pathogenic E. coli (one to two weeks), could be the actual cause for the exaggerated losses associated with H9N2 infections in commercial broilers.	Mahmoud et al.,2022

9.1.2 Domestic birds:

9.1.2.1 Chickens and turkeys

AIV represents a significant avian pathogen, causing substantial global economic losses (Salaheldin et al., 2022). In Egypt, where many domestic backyard geese and ducks remain unvaccinated, the presence of wild and domestic waterfowl introduces an additional concern. These waterfowl can act as carriers of highly pathogenic viruses, potentially spreading the infection even in the absence of noticeable symptoms (van den Brand et al., 2018; Caliendo et al., 2022).

In Egypt, a significant number of domestic birds are found in diverse production environments, such as large commercial farms, both industrial and integrated, along with non-regulated, non-registered small to medium-scale farms, including backyard farms linked to households. It's noteworthy that the non-regulated, non-registered small to medium-scale farms contribute to over 75% of the country's broiler production (Abdelwhab and Hafez, 2011). AI (H5N1) viruses have been identified in various hosts in Egypt, spanning chickens, ducks, quails, pigeons, ostriches, great egrets, crows, donkeys, pigs, wild birds, and humans (Kayed et al., 2019). The detection of HPAI H5N1 viruses in domestic birds in Egypt dates back to 2006 (Aly et al., 2008). Since 2008, HPAI H5N1 has been considered endemic in domestic birds. Genetic changes in the HA protein led to the co-circulation of genetic clades 2.2.1 and 2.2.1.1 from late 2009 to 2011. Clade 2.2.1.1 is believed to have emerged as a vaccine-escape mutant (Abdelwhab et al., 2016a). Clade 2.2.1 of HPAI H5N1 viruses continued to evolve, forming a new phylogenetic cluster named clade 2.2.1.2, which recently evolved into clade 2.2.1.2a (Arafa et al., 2015). In addition to the endemic status of HPAI H5N1 virus in poultry, Egypt experienced incursions of LPAI H9N2 viruses in 2010 (Naguib et al., 2015) **Figure 2**.

The HPAI H5N8 virus was recently identified in common coots during December 2016 in Egypt (Selim et al., 2017). Through analysis of their entire genome sequences, six genotypes of the HPAI H5N8 viruses were recognized in both migratory and domestic birds in Egypt (Anis et al., 2018; Yehia et al., 2018; Moatasim et al., 2019; Kandeil et al., 2022). The virus rapidly disseminated among domestic poultry throughout different areas in Egypt, presenting a substantial threat to the poultry sector (Kandeil et al., 2022). Recently, a distinct HPAI H5N2 reassortant virus was identified on a poultry farm in Beheira governorate, alongside a newly reassorted LPAIV H9N2 virus found in multiple chicken farms throughout Egypt (Hassan et al., 2020a). The emergence of the novel HPAI H5N2 virus in Egypt in 2018/2019 was attributed to reassortment events involving LPAI H9N2 and HPAI H5N8 strains (Hagag et al., 2019; Hassan et al., 2020a).

LPAI H9N2 viruses were identified in various avian species, including chickens, ducks, turkeys, quail, and pigeons, present in both commercial and backyard farms in northern Egypt (Monne et al., 2013). Despite the endemic nature of LPAI H9N2 in Egypt, the highest incidence of H9N2 in domestic birds in the Nile Delta occurred in January 2012, 2013, and 2014 (Naguib et al., 2015). Throughout the year, there is evidence of AIV detection in backyard poultry in Egypt, with infections in both backyard and commercial farms peaking during the winter and spring months (Salaheldin et al., 2018). Poultry infected with Egyptian H9N2 viruses typically exhibit either no clinical illness or mild respiratory signs (Kandeil et al., 2016). Active surveillance of AI among poultry in Egypt has revealed that co-infection with H9N2 and H5N1 within the same avian host is a common occurrence (Kayali et al., 2014). Simultaneous viral infections involving H5N1, H9N2, and H5N8 influenza viruses were identified in Egyptian layer and broiler chickens. This triple co-infection raises concerns about the possible set of epidemic avian influenza strains. Given the minimal similarities in genetic and antigenic properties between the viruses H5N8 and H5N1, there is a suggested need for adjustments in avian influenza vaccination strategies in Egypt, accompanied by rigorous biosecurity measures (Shehata et al., 2019).

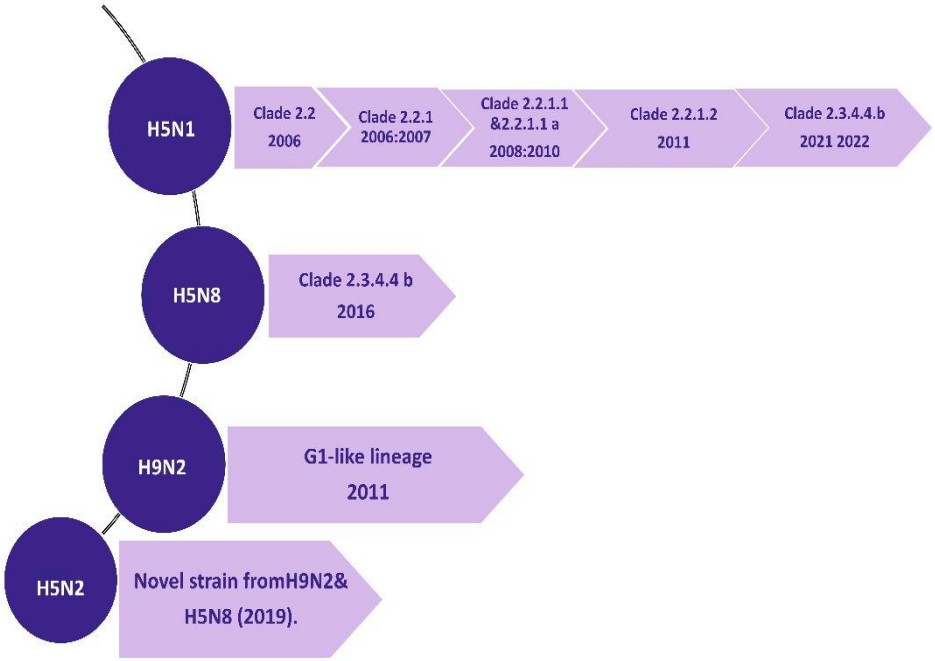

Figure 2: Avian influenza virus subtypes and their evolution in Egypt

9.1.2.2Waterfowls

H5N1

Domestic ducks serve as a vital intermediary in the transmission of H5N1 between wild waterfowl and domestic poultry, thus playing a pivotal role in shaping the epidemiology of the virus (Chen et al., 2004; Hulse-Post et al., 2005). The ability of domestic ducks to maintain the circulation of H5N1 HPAI viruses presents a public health risk, highlighting the importance of managing their ongoing circulation and transmission (Kim et al., 2009). The first known cases of H5N1 virus infection in domestic ducks in Asia occurred in 1997, and they were linked to live bird markets in Hong Kong (Shortridge et al., 1998). In 2002–2003, the first recorded outbreak of H5N1 virus causing mortality in ducks and geese occurred in Hong Kong. Meanwhile, infection with H5N1 in domestic poultry is known to result in high rates of mortality, typically ranging from 75% to 100% (Li et al., 2011). In general, AI viruses, including the highly pathogenic H5 and H7 strains, usually do not make ducks sick or cause death (Pantin-Jackwood et al., 2007). During an outbreak of HPAI virus in Italy in 2001, ducks were observed experiencing mortality from natural infection with AIV for the first time (Capua and Mutinelli,2001). Since then, several strains of H5N1 HPAI have been known to cause illness in ducks (Pantin-Jackwood et al., 2007; Löndt et al., 2010; Phuong et al., 2011). Clinical signs in affected ducks varied in severity, initially manifesting as passivity, ruffled feathers, conjunctivitis, and mild depression. As the disease progressed, ducks exhibited severe neurological symptoms such as torticollis, lack of coordination, tremors, cloudy eyes, and blindness. During postmortem examination, the only notable findings were congested lungs and liver (Bröjer et al., 2013). The persistent spread of HPAI H5N1 viruses in Egypt since 2006, affecting both backyard and commercial poultry farms, may increase the mortality rate among ducks (Aly et al., 2008). Despite the immune pressure induced by H5 virus vaccines, HPAI viruses can still spread among farms, possibly leading to their mutation and evolution (Abdelwhab et al., 2010). Recent findings have pointed to ducks playing a key role in both the spread and evolution of HPAI H5N1 viruses (Ibrahim et al., 2011; Kaoud et al., 2014). The isolation of two H5N1 HPAI viruses in Egypt in 2007 and 2008, both belonging to HA clade 2.2.1, demonstrated varying levels of pathogenicity in Pekin ducks. The 2007 virus exhibited moderate pathogenicity, while the 2008 virus was highly pathogenic, with both viruses causing clinical signs and mortality in ducks (Wasilenko et al., 2011). Recently, HPAI H5N1 clade 2.3.4.4b viruses were detected for the first time in domestic ducks from live bird markets in Egypt (El-Shesheny et al., 2023).

H5N8

The introduction of HPAI H5N8 viruses of GS/GD clade 2.3.4.4b into Egypt in November 2016 via wild birds led to additional complications, including their spread within the poultry industry (Hassan et al., 2020b). Later, the same lineage of virus was isolated from domestic ducks in 2017 (Anis *et al*., 2018). Egypt reported the presence of H5N8 AIV, closely related to the European H5N8 HPAI clade 2.3.4.4b, based on the sequences of the HA and NA (Yehia et al., 2018). Egypt became the third country in the Middle East to confirm the H5N8 clade 2.3.4.4b (CIDRAP, 2016). The AIVs were isolated from their natural reservoirs, including Charadriiformes and orders Anseriformes, which encompass domestic ducks (Alexander, 2007; Krauss et al., 2007). Domestic ducks have been identified as reservoirs for various avian influenza virus subtypes, facilitating reassortments and contributing to virus ecology, propagation, and the emergence of new AIV genotypes (Barber et al., 2010; Parvin et al., 2020).

11.1. Spreading between bird species:

Due to their long migration paths, wild ducks are natural reservoirs of the AI virus and aid in its transmission (Su et al., 2015). The virus can infect domesticated ducks and land birds through contaminated water supplies or food (Yamaji et al., 2020). Birds mostly transmit infections through the oral-fecal pathway and

H9N2

Domestic ducks have been recognized as reservoirs for multiple subtypes of avian influenza viruses, which enables reassortment and plays a role in the virus's ecology, spread, and the emergence of novel AIV genotypes (Barber et al., 2010; Parvin et al., 2020).

H5N2

A duck farm in Dakahlia governorate was the site of the 2019 discovery of a newly reassorted highly virulent H5N2 virus. This virus inherited seven gene segments from the extremely dangerous H5N8 virus and one gene segment from the less dangerous H9N2 virus, which encodes neuraminidase (N2) (Hagag et al., 2019).

9.1.2.3 Pigeon:

Pigeons, members of the Columbidae family, are commonly consumed in various countries, particularly homing pigeons. In 2013, a study found one of nine hospitalized H7N9-positive patients was working with poultry including pigeons (Li et al., 2014). In addition, healthy pigeons have tested positive for zoonotic low pathogenic avian influenza H7N9, suggesting they could serve as reservoirs for viruses causing infections in mammals (Abolnik, 2014). Unlike ducks & chickens, pigeons can be contaminated with AIV, including highly pathogenic strains, without displaying noticeable clinical signs (Abolnik, 2014). Pigeons, although infected with H5N8 HPAIV, may not show clinical signs, potentially making them healthy reservoirs of the virus (Kwon et al., 2017). Poultry in Egypt has tested positive for AI H9N2 viruses. This includes pigeons, quails, chickens & turkeys (Kandeil et al., 2019). A 2014 study identified novel reassortant H9N2 viruses in Egyptian pigeons, inheriting genes from both endemic H9N2 viruses and Eurasian AIVs in wild birds (Kandeil et al., 2017). Another study in Egypt isolated H5N1 virus from naturally infected pigeons, suggesting their susceptibility to H5N1 HPAIVs and their potential role as a carrier of pollution for additional avian species & humans (Mansour et al., 2014).

9.1.3 Infection of mammals:

AIV has been detected in various mammals. Examples include AI subtypes (H7, H3) in equines, AI subtypes (H3, H1) in swine, & AI subtypes in aquatic mammals (H7, H13, H10, and H4). These subtypes come from the genetic material characteristic of viruses normally present in waterfowl (H16–H1) (Wahlgren ,2011).

10. Reservoir and source of infection:

Waterfowl, including ducks, geese, and swans (Anseriformes), in addition to gulls & shorebirds (Charadriiformes), function as the ordinary reservoirs for the AI virus. These waterfowl comprise over 100 species from approximately twenty-five distinct families, demonstrating the worldwide spread of the virus among wild waterfowl (Lee et al., 2017a). Subtypes of avian IV, for example H5, H9, H7, & H6, can be found in both poultry & waterfowl (Capua and Alexander, 2004). The virus mostly reproduces in the epithelial cells of the digestive tract (Webster et al., 2007). Birds who are infected with the virus as well as not showing any symptoms typically release the virus into the environment through their droppings, saliva, in addition nasal secretions (Philippa et al., 2005). Other animals, such as swine, can also serve as sources of infection (Brown et al., 2007).

11. Mode of infection and transmission:

can stay infectious for about 21 days due to the large viral levels discovered in the faeces of afflicted poultry (Wahlgren, 2011; Bui et al., 2016). Proximity to water is a major risk factor for viral transmission because of the potential for close interactions amongst domestic poultry & migratory bird activities, which increases the

spread of disease (Chatziprodromidou et al., 2018). Moreover, the AI virus can be transmitted by body fluids & discharges for instance saliva, mucus, in addition to urine (Swayne, 2008). These fluids and waste products can contaminate many surfaces in production systems, including worker shoes and uniforms, cages, tools, and mechanical egg collection devices. It is believed that illnesses primarily transmit through this mechanism among flocks (Caría et al., 2017). blaming industrial poultry for the majority of reported worldwide outbreaks (Horimoto and Kawaoka,2001).

11.2 Transmission to mammals:

As there is no evidence supporting the transmission of the virus to mammals through aerosols, direct contact remains the primary mechanism of transmission for mammals **Figure 3**. (Herfst et al., 2018). AI viruses have the capacity to adapt and disseminate, as evidenced by their ability to transfer from infected birds living in dense populations to other species (Yamaji et al., 2020). The virus needs to adjust & modify itself to the new host to spread extensively as well as multiply in animals. Almost all significant disease outbreaks in the past have been caused by a process known as viral reassortment (Gambaryan and Matrosovich, 2015; Cáceres et al., 2021). Mice, pigs, and cats have all contracted the H5N6 strain of the avian flu virus (Cao et al., 2017). There have been reports of H3N8 subtype infections in dogs (Yoon et al., 2005). Although tigers and leopards have been found to harbor the H1N1 subtype (Amonsin et al., 2006). There is a connection between these cases and avian influenza outbreaks. Furthermore, by exposing ferrets and other animals to several avian influenza subtypes in experimental settings, researchers have examined how dangerous they are (Kwon et al., 2018).

11.3 Zoonotic transmission:

Avian flu viruses can spread to other species, including bats, for a variety of reasons that facilitate transmission (Nabi et al., 2021); pigs (Zhang et al., 2020); horses; cats; ferrets; sea lions; and bats (Roguski and Fry ,2019) ; these species can also act as reservoirs for the viruses that infect humans and birds, allowing for genetic mixing (To et al., 2013). Several variables come together to create the ideal setting for the spread of avian influenza to humans, including host susceptibility, favourable environmental circumstances, viral mutations, and contact with infected birds (Chan et al., 2013; WOAH, 2023b).

The main way that the virus spreads among humans & birds is through close connection with the stool or discharge of diseased birds as well as exposure to infected settings (WOAH, 2023c). Human-to-human transmission is not supported by any evidence (Yamaji et al., 2020). Workers in poultry farming and processing have a higher risk of infection than the public owing to extended contact to the virus (WOAH, 2023d).

12. Conclusion:

We clarified the fundamental compositions, activities, and roles of avian influenza proteins in the progression of disease and viral pathogenesis in this review. We also investigate the probability of co-infection by bacteria, viruses, & AIV to maximize the functionality and operation of the virus and so boost pathogenicity. As a result, this accelerates the disease's spread and increases its difficulty in controlling. It has consequently become one of Egypt's most serious viral infections, harming chickens and having disastrous financial effects.

13.References

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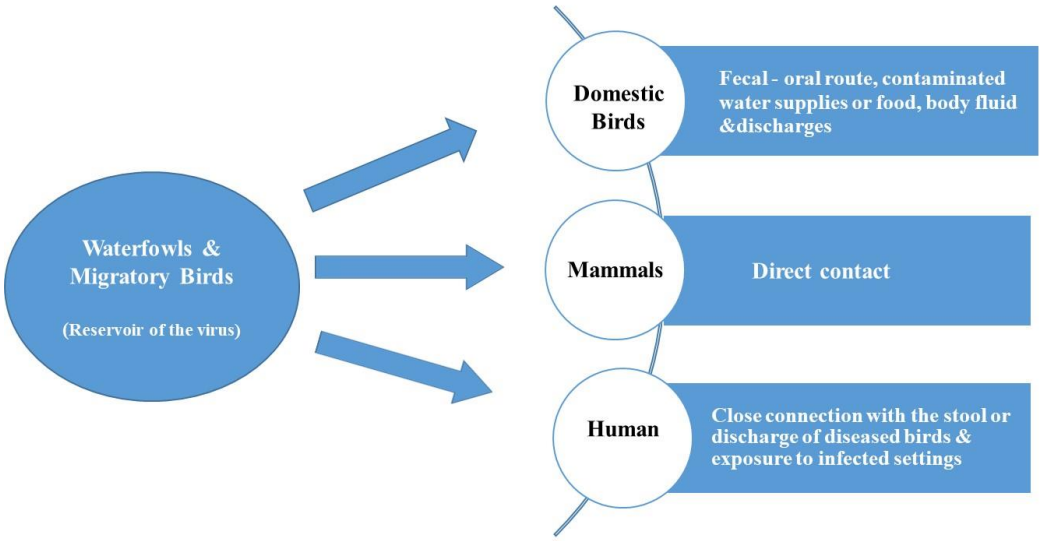

Figure 3. Routes of transmission of avian influenza virus

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